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TITLE: CTLA-4 Blockade-Based Immunotherapy in Prostate Cancer

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Background: CTLA-4 is an inhibitory molecule on T cells that induces T cell downregulation. Granulocyte-macrophage colonystimulating factor (GM-CSF) is a growth and survival factor for dendritic cells. The safety of combining GM-CSF with CTLA-4 blockade in prostate cancer patients is being investigated in an ongoing phase I trial.

Methods: Sequential cohorts of 3-6 patients receive GM-CSF 250 μg/m2/d subcutaneously on days 1-14 of a 28-day cycle with escalating doses of anti-CTLA antibody on day 1 of each cycle x 4. Patients are monitored for clinical autoimmunity with T cell phenotyping performed.

Results: Twelve patients have been treated to date. Dose-limiting toxicity (DLT) was not observed in the initial CTLA-4 antibody dose level. Two DLTs, consisting of a vertebrobasilar TIA possibly related to therapy and a generalized rash requiring steroids were observed in the second and third dose levels respectively, resulting in expansion of each to 6 patients. No laboratory evidence of autoimmunity has been observed in any patient. Expansion of monocytes / dendritic cells and upregulation of PBMC activation markers have been seen, consistent with known GM-CSF effect.

Conclusions: CTLA-4 blockade and GM-CSF has demonstrated preliminary safety in advanced prostate cancer. Accrual and immunologic analyses are ongoing.

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Introduction

This research project ultimately aims to develop effective immunotherapy for prostate cancer. Specifically, we are exploring the use of Granulocyte Macrophage-Colony Stimulating Factor (GM-CSF) in combination with blockade of a T cell inhibitory molecule called Cytotoxic T-Lymphocyte-Associated Molecule-4 (CTLA-4). We are initially studying repetitive dosing of an anti-CTLA-4 antibody in combination with subcutaneous GM-CSF to determine the safety of this combination for future clinical testing. We will then move this combination therapy into a phase II trial to look at effects on PSA and other clinical endpoints. Concomitantly, peripheral blood is collected from patients to evaluate the immune response generated.

Body

With regard to the original proposed statement of work, task 1 was to determine if polyclonal T cell activation and clinical autoimmunity occur when CTLA-4 blockade is combined with GM-CSF. We have initiated a phase I trial with this combination in men with hormone refractory metastatic prostate cancer. We have collected samples from these men at baseline and throughout therapy, and have begun to determine relative numbers of activated T cells and other immune markers. Patients have been monitored for clinical signs and symptoms of autoimmunity, and initial presentations of this data in abstract form have been made. Task 2 or 3 from the original Statement of Work have not yet been addressed, pending outcome from the phase I trial, as described under Task 1. We have treated a total of 12 men on the phase I trial, and have observed two dose-limiting toxicities possibly attributed to treatment. One was a cerebral vascular event and the other was a rash. Both of these events resolved upon discontinuation of therapy.

No clear laboratory evidence of autoimmunity has been observed in this first cohort of men. In addition, initial immunologic results are available, as seen in the accompanying figures (Figure 1 and Figure 2). Monocytes, and to a lesser degree, dendritic cells, were increased with this combination immunotherapy. This expansion was associated with an up-regulation of activation markers CD25 and CD69. Given that this is a single-arm study, the differential effect of the two agents on these parameters is not able to be surmised. Further, after 14 days of treatment a slight up-regulation of CD4-positive T cells bearing the activation marker CD69 and CD25 was observed. No observable differences in CD45 RA or CD45 RO were observed in any T cell subset. Last, as a general read-out of the ability of therapy to stimulate immunity, CMV reactive T cells as detected by MHC peptide tetramers were measured. As observed in Figure 3, the percentage of CMV-positive T cells increased

with treatment. Of note, these T cells expressed the activation markers CD69 and CD107A, as well as demonstrated production of interferon-gamma

Key Research Accomplishments

1. Anti-CTLA-4 antibody and GM-CSF have demonstrated preliminary safety when given in combination to metastatic hormone refractory prostate cancer patients.

2. Collection and immunologic assessment of baseline and serial peripheral blood samples is feasible.

3. This combination immunotherapy in metastatic hormone refractory prostate cancer patients produces expansion of activated monocytes and dendritic cells, as well as activation of an endogeneous population of cytotoxic T-lymphocytes *in vivo*.

Reportable Outcomes

1. This data was a poster presentation at the ASCO 2004 annual meeting (L. Fong, B. Rini, B. Cavanaugh, E. Small. CTLA-4 Blockade-Based Immunotherapy for Prostate Cancer. Proc Am Soc Clin Oncol 22:14s, 2590, 2004).

2. This data was presented at the National Specialized Program of Research Excellence (SPORE) meeting in Baltimore, MD in July 2004. It was an oral presentation.

3. A serum repository of baseline and treatment samples for all patients is available and stored in the Immunology Core Laboratory of Dr. Larry Fong, who is performing the immunologic assays. This repository of serum will provide valuable companion data to this study, and a potential source of data for future studies.

4. On the basis of this preliminary work and funding through the DOD, Dr. Rini has applied for a Career Patient-Oriented Investigator Award (K23) which has received a score of 140, A funding decision is pending.

Conclusions

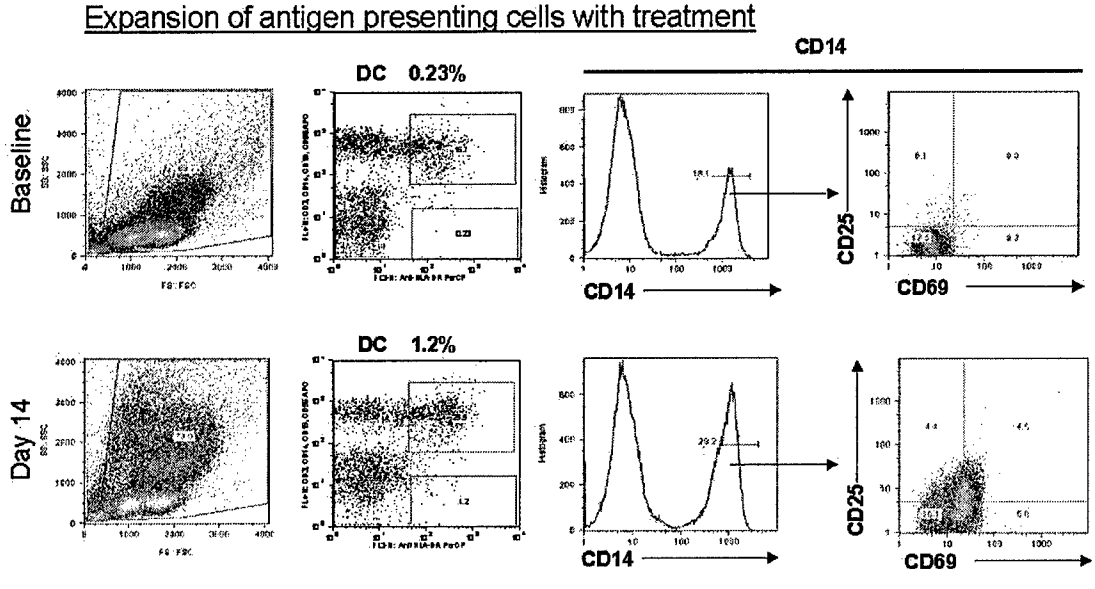
CTLA-4 blockade-based immunotherapy in combination with GM-CSF is feasible in metastatic hormone refractory prostate cancer. Without substantial clinical autoimmunity to date, further accrual to the phase I portion of this study will define the maximum tolerated dose of this combination for future clinical testing in prostate cancer. Initial immunologic results suggest an effect of this therapy on both monocytes, dendritic cells, and T cells. Correlation of this response with clinical outcome is forthcoming.

References

Fong L, Rini B, Cavanaugh B, Small E. CTLA-4 blockade-based immunotherapy for prostate cancer. Proc Am Soc Clin Oncol 22:14s, 2590, 2004.

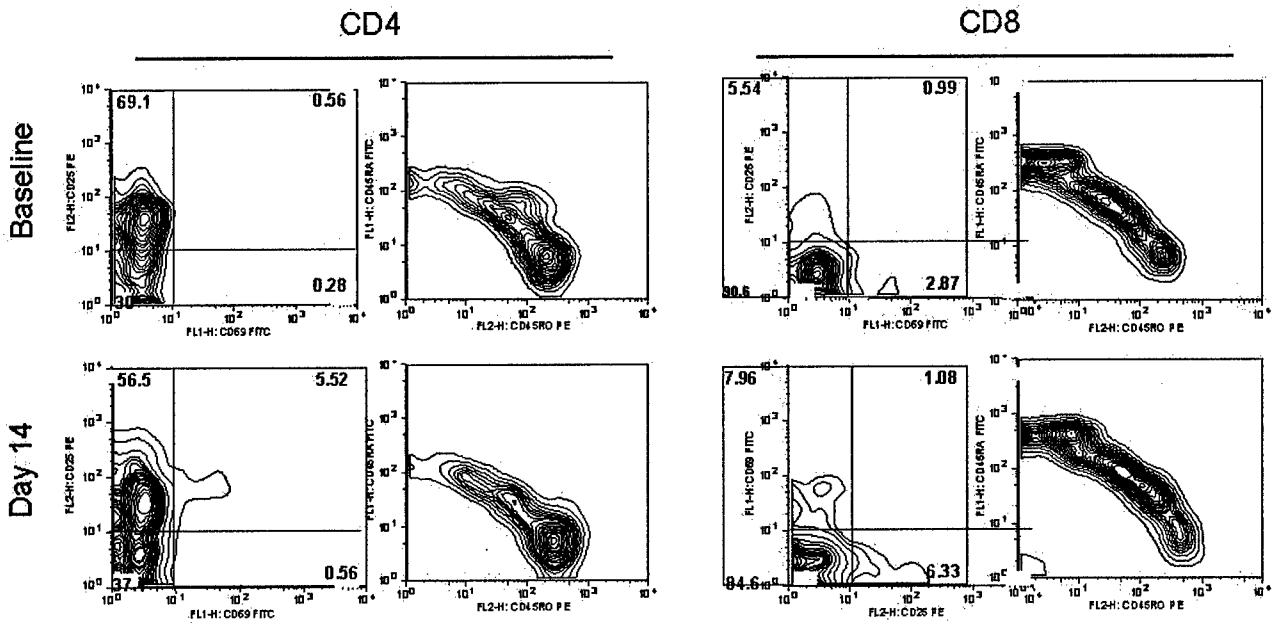
Appendices

Figure 1



- Monocytes and, to a lesser degree, dendritic cells were increased with treatment.
- This expansion was associated with upregulation of activation markers CD25 and CD69.
- This effect is similar to that seen with GM-CSF treatment alone.

Figure 2



After 14 days of CTLA-4/GM-CSF therapy, a slight upregulation of cells double positive for the T cell activation markers CD69 and CD25 was seen in bulk CD4 cells. Whereas in bulk CD8 cells a slight upregulation in CD25 alone was seen. No observable differences could be seen in the coexpression of CD45RA and CD45RO in bulk CD4 or CD8 cells at this time point.

Figure 3

In vivo modulation of antigen-specific $CD8^+$ T cells with treatment.

• In patients with CMV reactive T cells detectable by MHC/peptide tetramers, this treatment led to the upregulation of CD69 as well as the functional markers CD107a and INFγ consistent with activation of the T cells *in vivo* by the treatment.